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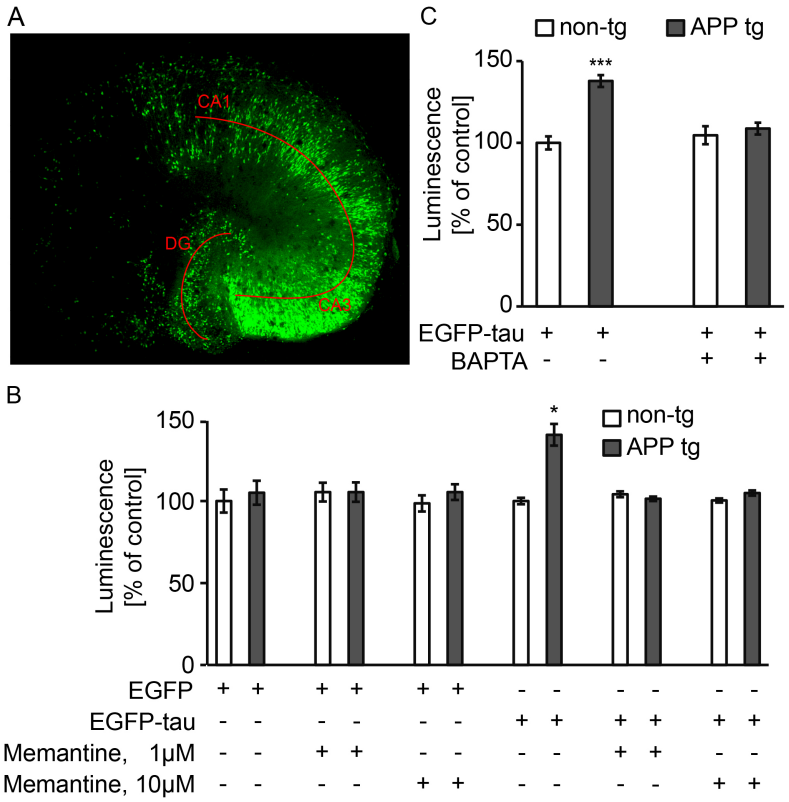
The β-amyloid peptide (Aβ) and tau are key molecules in Alzheimer's disease causing neuronal dysfunction and cell death. Evidence exists that tau mediates the pathology downstream of Aβ. N-methyl-D-aspartate receptors (NMDARs) are supposed to play an essential role in the pathophysiology of Aβ and tau. However, the exact mechanisms deciphering how Aβ could induce tau-dependent neuronal dysfunctions are still unclear. Here we show that virus-mediated expression of human tau causes neuronal degeneration in organotypic hippocampal slice cultures from APP transgenic mice but not in non-transgenic cultures. Treatment with therapeutically concentrations of NMDAR open channel blocker memantine completely abolished tau-dependent cell death in APP transgenic cultures. Removing extracellular calcium with calcium chelator BAPTA also prevented tau toxicity. Our data indicate that human tau-dependent neuronal cell death in APP transgenic slice cultures is mediated by calcium influx through NMDARs.

Objective

It has been shown that synaptic loss in APP transgenic slice cultures occurred via NMDA receptor signaling but independent of calcium flux. Here, we aim to determine whether neuronal tau-dependent cell death in the same cultures also depends on calcium flux-independent NMDA receptor activity.

Introduction

Alzheimer's disease (AD) is characterized by aggregates of the Aβ and tau. Evidence exists that tau mediates pathological events downstream of Aβ **(Rapoport 2002[1]) (Roberson 2007[2]) (Ittner 2010[3])**. NMDARs play an essential role for Aβ-induced neuronal dysfunction and inhibition of NMDARs protects against Aβ-induced synaptic loss **(Tackenberg 2009[4]) (Shankar 2007[5])**. Furthermore, the effect of Aβ on tau is mediated by NMDARs as blocking glutamate-binding to NMDARs prevents the induction of a tau-dependent neuronal cell death in APP transgenic cultures **(Tackenberg 2013[6])**. NMDARs can modulate intracellular signaling cascades via calcium influx into the cell. However, metabotropic NMDAR signaling, i.e. independent of ion flux, has also been described **(Nabavi 2013[7]) (Tamburri 2013[8])**. It has been recently shown that oligomeric Aβ caused synaptic loss in hippocampal slice cultures via NMDAR signaling but independently of calcium influx **(Birnbaum 2015[9])**. In the present study we investigated, whether the tau-dependent neuronal cell death in APP transgenic cultures depends on ionotropic (calcium influx) or metabotropic NMDAR functions.



Tau toxicity in APP transgenic slice cultures is blocked by memantine and BAPTA treatment. **A:** Representative image of a hippocampal slice infected with EGFP-tau expressing Sindbis virus. **B:** Cytotoxicity assay of EGFP or EGFP-tau expressing slices from non-transgenic (non-tg) or APP transgenic (APP tg) mice. Slices were treated with 1 µM or 10 µM memantine. Memantine, at both, concentrations prevented the toxicity of human tau in APP tg cultures. n=5 **C:** Cytotoxicity assay of EGFP-tau expressing non-tg and APP tg slices treated with 2 mM of calcium chelator BAPTA or BAPTA solvent $NaHCO_3$ (-). BAPTA treatment abolished tau toxicity in APP tg slices. n=8;
Values are shown as mean ± SEM; * p = 0,019 (B), *** p = 0,00009 (C), two-tailed paired Student's t-test.

Results & Discussion

Results

We prepared organotypic hippocampal slices from APP transgenic mice and non-transgenic littermates and expressed EGFP (control) or EGFP-coupled human 441 tau selectively in neurons using neurotropic Sindbis virus. After infection EGFP fluorescence was observed in all hippocampal subregions, i.e. dentate gyrus (DG) as well as in the *cornu ammonis* areas with the strongest signals in CA3 (Fig. 1A).

In organotypic slices from APP transgenic mice Aβ induced a human tau-dependent cell death **(Tackenberg 2013[6])**. Here, we first confirmed this data by showing that EGFP-tau expression increased cytotoxicity assay luminescence in APP transgenic compared to non-transgenic cultures (Fig. 1B; n = 5, p = 0,019, two-tailed paired Student's t-test). Considering slight variations in infection efficiencies between different cultures and experiments, we normalized cytotoxicity assay luminescence from each culture to the EGFP / EGFP-tau signal, measured in the lysate from the respective culture.

In contrast to EGFP-tau expressing neurons, no toxicity was observed in APP transgenic cultures upon expression of EGFP alone (Fig.1B). This suggests that transgenic expression of APP and its cleavage products, such as Aβ, do not cause cell death *per se* but require the presence of human tau. To determine whether tau toxicity in APP transgenic cultures was mediated by ion flux through NMDA receptors, we first treated cultures with clinically relevant concentrations (1 µM and 10 µM, according to **(Xia 2010[10])**) of the NMDAR open channel blocker memantine. 1µM Memantine has been shown to block synaptic transmission in organotypic slices **(Birnbaum 2015[9])**. At both concentrations, memantine treatment completely abolished tau-dependent cell death in APP transgenic cultures (Fig. 1B). To corroborate that the influx of calcium through the NMDAR is an essential process in this event, we treated slices with 2 mM of non-cell-permeable calcium chelator BAPTA or its solvent $NaHCO_3$ as control. BAPTA treatment completely abolished tau toxicity in APP transgenic cultures indicating that tau-dependent cell death requires calcium flux into the neuron.

Discussion

We showed that human tau expression in APP transgenic but not in non-transgenic cultures causes neuronal cell death. The induction of tau toxicity in these cultures can be blocked by applying anti Aβ antibodies **(Tackenberg 2013[6])**, indicating that Aβ and not APP or any other APP cleavage product confers toxicity to human tau. We further provide evidence that calcium influx through NMDARs is required for this process. In contrast, previous studies showed that Aβ-induced synaptic loss did not depend on calcium influx **(Birnbaum 2015[9])** and did not depend on the presence of tau **(Tackenberg 2013[6])**. This indicates that Aβ-induced loss of synapses and neuronal cell death are two independent events, induced by distinct pathways. Although our data show that tau-dependent toxicity in APP transgenic cultures requires NMDAR-mediated calcium flux, it remains to be shown whether calcium influx is an event upstream or downstream of tau toxicity. Ittner and colleagues proposed a model in which the presence of tau is required for Aβ-induced excitotoxicity, which supports a role of calcium flux downstream of tau **(Ittner 2010[3])**. In contrast, pharmacological activation of extrasynaptic NMDARs – even in the absence of Aβ – increased phosphorylation and toxicity of tau **(Tackenberg 2013[6])**. This suggests a role of calcium influx upstream of tau. Thus, it will be important to analyze whether the treatment with memantine or BAPTA prevents aberrant posttranslational modifications of tau, such as increased phosphorylation. This would confirm a role of calcium flux upstream of tau. These experiments could be performed in a follow-up study.

Additional Information

Methods and Supplementary Material

Please see https://sciencematters.io/articles/201602000028.

Ethics Statement

All animal experiments were performed in accordance with the guidelines of the Swiss veterinary cantonal office.

Citations

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